CAUSES FOR DECREASED FERTILITY IN OUT-OF-SEASON MATED EWES

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ABSTRACT

The interval from onset of estrus to preovulatory luteinizing hormone (LH) release, conception and fertilization rates, and number of accessory spermatozoa per ovum at 48 hr postmating in untreated cyclic ewes and in progestogen-pregnant mare serum gonadotropin (PMSG) treated, anestrous ewes were compared in efforts to identify sources of lowered fertility for matings induced in anestrous ewes with exogenous hormones. Blood samples for LH determination were collected at 0, 2, 4, 6, 8, 10, 12, and 24 hr after the onset of estrus. Conception and fertilization failure rates were determined at 48 hr, 12 days, or parturition. The progestogen-PMSG treated ewes had a shorter interval from onset of estrus to preovulatory LH release, lower conception rates, and fewer accessory spermatozoa than cyclic ewes had. Conception failure, rather than embryonic mortality, was the major cause of reduced fertility for the out-of-season mated ewes and apparently resulted from insufficient viable spermatozoa in the oviducts to fertilize the ova.

INTRODUCTION

Many studies have been conducted to determine the feasibility of inducing fertile matings in seasonally anestrous ewes by the use of exogenous hormones. The treatment of anestrous ewes with progestogens followed by pregnant mare serum gonadotropin (PMSG) has resulted in a high percentage of ewes showing behavioral estrus but only moderate conception rates (1-3). The objective of this experiment was to identify the source and physiological basis for the lowered conception rates of anestrous ewes after hormone therapy.

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MATERIALS AND METHODS

Spermatozoa transport, fertilization rate, embryo survival, and the relationship between onset of estrus and the occurrence of the preovulatory LH peak in 30 cyclic Finnsheep-cross (3/4) and in 30 progestogen-PMSG treated, anestrous Finnsheep-cross (3/4) ewes (out-of-season mated) were compared. The progestogen-PMSG treatment consisted of an intravaginal, progestinated pessary, containing 20 mg of flurogestone acetate (synchro-mate, G.D. Searle and Co., Skokie, Illinois), inserted into the anterior vagina for 9 days, followed by an intramuscular injection of 20 mg of progesterone in corn oil and 500 IU of PMSG in sterile saline at pessary removal. The untreated, control ewes (cyclic ewes) were naturally mated during the normal breeding season. A vasectomized ram and a fertile ram were penned with the ewes 3 days before expected estrus. Both a vasectomized and fertile ram were penned with the ewes to improve estrous detection, but yet allow each ewe to be mated to the same two fertile rams an equal number of matings. The ewes were continuously observed for estrus starting 2 days before expected estrus and continued until all ewes were detected in estrus. At the onset of estrus, each ewe was mated to two fertile rams twice within 1 hr. An evaluation of semen from each ram indicated greater than 60% normal acrosome morphology and 2 x 10^9 spermatozoa/ejaculate.

Immediately after an ewe was detected in estrus, a 10-ml sample of jugular vein blood was collected by venipuncture for LH determination; additional blood samples were collected at 2, 4, 6, 8, 10, 12, and 24 hr after the onset of estrus. Blood was collected into heparinized syringes, refrigerated, and centrifuged, and the plasma was stored at -10° C until assayed. Plasma LH concentration was determined by a double antibody radioimmunoassay (4) and is expressed as nanograms of NIH-LH-B8/ml of peripheral plasma.

Ewes were assigned to one of three embryo recovery groups after being mated by two fertile rams: 1) embryos surgically recovered at 48 hr postmating; 2) embryos surgically recovered at 12 days postmating; and 3) lambs obtained at parturition. Each ewe in the 48-hr recovery group was anesthetized 48±1 hr after the first mating by a fertile ram, and the oviducts and the anterior two-thirds of the uterine horns were exposed through a mid-ventral laparotomy. A Teflon cannula was inserted into the infundibular end of the oviduct and anchored with a nylon suture; the free end of the cannula was placed in a collection dish. A 20-guage hypodermic needle was inserted into a uterine horn 1-2 cm posterior to the utero-tubal junction, the uterine horn lumen was clamped immediately posterior to the needle, and the oviduct was flushed alternately with 3 ml of modified Brinster's medium (BMOC-3) and 3 ml of air twice. Percentage of ova recovered at 48 hr postmating relative to number of corpora lutea was 92.3%. Ova were examined by phase contrast microscopy for cleavage and the presence of spermatozoa in the zona pellucida (accessory spermatozoa). The 1-cell ova were cleared for 24 hr in 25% glacial acetic acid in ethanol (25% V/V) and stained with 1% natural orcein (W/V) to identify pronuclei. Number of ova recovered, number of ova fertilized, and number of accessory spermatozoa were recorded.

The oviducts, uterus, and cervix were surgically removed from the ewes in the Day 12-recovery group 12 days postmating. The uterine horn was clamped and severed at the uterotubal junction. A second clamp was applied to the contralateral uterine horn adjacent to the uterine body, after which the uterine body and one horn were flushed together with the alternate introduction of 50 ml of saline and 20 ml of air three times. The uterus was massaged during each saline infusion, the flushings and embryos were expelled from the anterior end of the uterine horn, and the number of embryos per horn was recorded. The flushing procedure was repeated for the opposite horn. Ewes in the lambing group were individually penned before parturition, and the number of lambs born per ewe was recorded.

Data involving plasma LH concentration, number of accessory spermatozoa, and time of LH release were analyzed by analysis of variance (5). Differences in number of embryos among recovery groups were compared by the Mann-Whitney U-test (6). Conception rates were compared by the Chi-square test (5).

RESULTS AND DISCUSSION

Timing of the maximal preovulatory LH release in relation to onset of estrus is illustrated in Figure 1. The interval from the onset of estrus to maximal preovulatory plasma LH concentration was shorter ($P < .01$) for the out-of-season mated ewes than for the cyclic ewes. About 70% of the out-of-season mated ewes showed maximal LH concentration within 0 to 2 hr after onset of estrus, whereas 73.3% of the cyclic ewes had the maximal concentration at 10 to 12 hr after onset of estrus. The extent to which the LH release preceded onset of estrus in the out-of-season mated ewes could not be evaluated, because the blood samples were collected at the onset of estrus. Ova collected from the out-of-season mated ewes at 48 hr after the onset of estrus were at the 2- to 4-cell stage; ova collected from the cyclic ewes were at the 1-cell with 2 pronuclei stage. The stage of recovered ova suggests that the interval from onset of estrus to ovulation was decreased by about 12 hr, the time required for the division from a 2-cell to a 4-cell embryo (7), in the out-of-season mated ewes. Similarly, ewes treated during the normal breeding season with flurogestone acetate-impregnated pessaries had an earlier LH release in relation to onset of estrus than untreated cyclic ewes had (8). The decreased interval from onset of estrus to LH peak and ovulation for the out-of-season mated ewes may result in fewer spermatozoa reaching the oviducts and may account for the fewer number of accessory spermatozoa in out-of-season mated ewes. Previous reports indicate that the altered relationship between onset of estrus and LH release associated with flurogestone acetate was limited to this compound and was not observed with progesterone or other synthetic progestogens (4,8). Magnitude of the LH release was not affected by treatment.

A comparison of conception rate, fertilization rate, and number of accessory spermatozoa per ovum for cyclic ewes and out-of-season mated ewes is presented in Table 1. The overall conception rate was 100% for the cyclic ewes and 76.6% for the out-of-season mated ewes; these rates

are equal to or higher than those previously reported for similar ewes (1-3). The relatively high conception rates in this study for one estrus, especially in the control ewes, may have resulted from the individual mating of each ewe to two rams. Fertilization rate was expressed as the percentage of fertilized ova or blastocysts to corpora lutea for only the pregnant ewes and was not affected ($P>.05$) by treatment. The decrease in fertilization rate between 48 hr and 12 days postmating for the out-of-season mated ewes was not significant ($P>.05$). Thus, failure to conceive, rather than embryonic mortality, appears to be the source of reduced fertility for out-of-season mated ewes. The fact that the out-of-season mated ewe had a greater ($P<.05$) number of corpora lutea than the cyclic ewes indicates that the administration of 500 IU of PMSG or the season, or both, resulted in increased ovulation rate.

Spermatozoa were observed in the oviductal flushings and the zona pellucida of ova from only those ewes with fertilized ova at 48 hr postmating. The number of spermatozoa in the zona pellucida of the fertilized ova was fewer ($P<.01$) for the out-of-season mated ewes than for the cyclic ewes (5.5 vs 79.1). The decreased number of accessory spermatozoa for the out-of-season mated ewes may have resulted from the shorter interval from the onset of estrus to ovulation, altered uterine motility associated with progestogen-regulated estrus (9), reduced spermatozoa viability, or a combination of these factors. The scarcity or absence of accessory and oviductal spermatozoa in the out-of-season mated ewes suggest that the lower conception rates for these ewes were the result of insufficient viable spermatozoa in the oviducts to fertilize the ova, as has been reported for progestogen-synchronized cyclic ewes (10). Data from this study indicate that the combination of prolonged progestogen treatment with a subsequent injection of PMSG for anestrous ewes does not alleviate the adverse side effects of prolonged progestogen treatment alone (4,8,9).

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Table I: Conception Losses in Naturally Bred Cyclic and Out-of-Season Mated Ewes[a]

Treatment	No. of ewes	No. of corpora lutea	No. of embryos[b]	Fertilization rate (%)[c]	Accessory spermatozoa/ ovum
Cyclic ewes (Control)					
48 hr	10	26	21(10)	80.5	79.1
12 days	10	32	30(10)	93.8	--
Parturition	10	--	22(10)	--	--
Out-of-season mated ewes[d]					
48 hr	10	52	32(6)	89.5	5.5[e]
12 days	10	39	20(8)	66.7	--
Parturition	10	--	19(9)	--	--

[a]Conception losses were determined at 48 hr postmating, 12 days postmating, or parturition.

[b]Number of ewes with either fertilized ova, blastocysts, or lambs are listed in parenthesis. Recovery rate for ova was 92.3%.

[c]Percentage of fertilized ova or blastocysts to corpora lutea for ewes with at least one embryo.

[d]Anestrous ewes (May) treated with flurogestone acetate pessary for 9 days plus 20 mg of progesterone and 500 IU of PMSG im at pessary removal.

[e]Out-of-season mated ewes had significantly fewer ($P < .01$) accessory spermatozoa/ovum at 48 hr than cyclic ewes had.

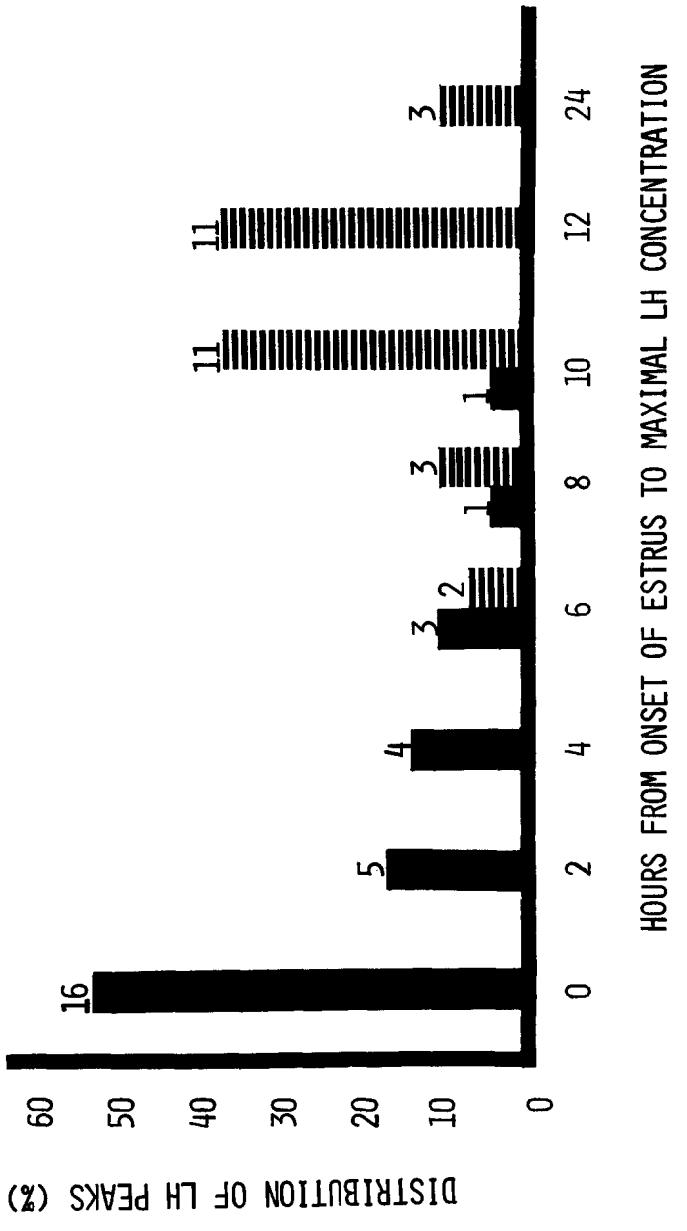

Figure 1: Percentage of cyclic ewes (▒) and progestogen-PMSG treated, anestrous ewes (■) with their maximal plasma LH concentration occurring at the indicated times after onset of estrus. The number of ewes with an LH peak is listed at the top of the bar.